IS 12014-2 (1986): Methods for Determination of Organic Preservatives in Foodstuffs, Part 2: Propionic Acid and Its Salts [FAD 16: Foodgrains, Starches and Ready to Eat Foods]

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IS : 12014 ( Part 2 ) - 1986
(Reaffirmed - 2012)

# *Indian Standard*

# METHODS FOR DETERMINATION OF ORGANIC PRESERVATIVES IN FOODSTUFFS

## PART 2 PROPIONIC ACID AND ITS SALTS

UDC 664·8/9 : 543 [ 547·293 ]



BUREAU OF INDIAN STANDARDS
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NEW DELHI 110002

Gr 3

*August* 1987

# *Indian Standard*

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## PART 2 PROPIONIC ACID AND ITS SALTS

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# *Indian Standard*

# METHODS FOR DETERMINATION OF ORGANIC PRESERVATIVES IN FOODSTUFFS

## PART 2 PROPIONIC ACID AND ITS SALTS

## 0. FOREWORD

**0.1** This Indian Standard ( Part 2 ) was adopted by the Indian Standards Institution on 30 October 1986, after the draft finalized by the Food Hygiene Sectional Committee had been approved by the Agricultural and Food Products Division Council.

**0.2** For protecting food from microbial deterioration, a number of methods, such as application of heat or cold, dehydration, fermentation irradiation or addition of certain chemicals, are employed. Besides extending the period of use of a food, a chemical preservative should be safe for human consumption, should not impart undesirable organoleptic changes, be economical in use and be capable of being analysed. While the use of chemical preservatives to be safe under conditions of use is governed by law, it is considered necessary to prescribe methods for their analysis. The use of these methods would not only ensure repeatable and reproducible results for their correct interpretation, but would also facilitate inter-laboratory comparisons.

**0.3** The prevention of Food Adulteration Act, 1954 and the rules framed thereunder allow the use of two classes of preservatives, Class I and Class II. Class I preservatives include common salt, sugar, dextrose, glucose ( syrup ), wood smoke, spices, vinegar or acetic acid, honey, etc. Class II preservatives include inorganic substances like sulphurous acid including salts thereof, nitrates of sodium or potassium, and organic substances like benzoic acid including salts thereof; sorbic acid including its sodium, potassium and calcium salts and sodium and calcium propionate.

**0.4** This standard covering the determination of organic preservatives is being issued in three parts. This part ( Part 2 ) covers the determination of propionic acid and its salts in foodstuffs. The Part I covers benzoic acid and its salts and Part 3 covers sorbic acid and its salts.

0.5 In reporting the results of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS : 2 - 1960*.

## 1. SCOPE

1.1 This standard ( Part 2 ) prescribes the methods for determination of propionic acid and its salt used as preservatives in foodstuffs.

## 2. QUALITY OF REAGENTS

2.1 Pure chemicals and distilled water ( *see* IS : 1070 - 1977† ) shall be employed in tests.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the results of analysis.

## 3. GENERAL

3.1 This standard specifies two methods for the determination of propionic acid and its salts, namely, paper and column chromatographic methods. Paper chromatographic method shall be used for qualitative detection and chromatographic method shall be used for qualitativ estimation of propionic acid and its salts.

## 4. PAPER CHROMATOGRAPHIC METHOD

### 4.1 Reagents

4.1.1 *Mobile Solvent* — Take two parts of acetone, one part of tertiary butyl alcohol, one part of n-butyl alcohol and one part of liquid ammonia and mix them. This solvent should always be prepared fresh.

4.1.2 *Chromogenic Reagent* — Add 200 mg each of methyl red and bromothymol blue to a mixture of 100 ml formalin and 400 ml absolute alcohol. Adjust to *p*H 5·2 with 0·1 N sodium hydroxide.

4.1.3 *Sodium Hydroxide* — 0·1 and 1N.

4.1.4 *Phosphotungstic Acid* — 20 percent solution in distilled water.

4.1.5 *Crystalline Magnesium Sulphate* — $MgSO_4 . 7H_2O$.

4.1.6 *Sulphuric Acid* — 1N.

4.1.7 *Propionic Acid Standard Solution* — Pipette 1 ml of propionic acid into a 100-ml volumetric flask and dilute to volume with distilled

*Rules for rounding off numerical values ( *revised* ).
†Specification for general laboratory use ( *second revision* ).

water. Pipette 1 ml of this stock solution into a 25-ml beaker and neutralize the acid with 0·1 N sodium hydroxide using cresol red indicator avoiding excess alkali. Evaporate to 0·5 ml in a water-bath.

**4.1.8** *Congo Red Indicator Paper*

**4.2 Apparatus**

**4.2.1** *Chromatographic Tank*

**4.2.2** *Pipettes* — with 0·1 ml graduations.

**4.2.3** *Chromatographic Paper* — Whatman No. 1, 20 × 20 cm, sheets.

**4.2.4** *Steam Distillation Apparatus*

**4.2.5** *Beakers* — 25-ml capacity.

**4.3 Procedure**

**4.3.1** *Sample Preparation*

**4.3.1.1** *All types of bread not containing fruit* — Take one or half loaf of bread and cut it into slices of 2-3 mm thickness. Spread the slices on the paper and let them dry in a warm room until sufficiently crisp and brittle to grind well. Grind entire sample to pass through 850 micron sieve; mix well and keep in an air-tight container before proceeding for experimentation.

**4.3.1.2** *Bread containing raisins and fruits* — Proceed as in **4.3.1.1** except comminute by passing twice through food chopper instead of grinding and dry air dried sample in an uncovered dish for 16 hours at 70°C under pressure of less than 50 mm of mercury.

**4.3.2** *Distillation* — Weigh accurately 10 g of air dried bread and transfer it to a 150-ml distillation flask. Add 40 ml distilled water and 10 ml of 1 N sulphuric acid, mix thoroughly and add 10 ml of 20 percent phosphotungstic acid solution. Mix the contents well and add 40 g magnesium sulphate. Swirl the contents well and make the solution acidic to congo red paper with 50 percent sulphuric acid. Connect the condenser and steam generator and distil 200 ml in 35 - 40 minutes. Immediately neutralize the distillate using cresol red and 0·1N sodium hydroxide. Evaporate the solution to 0·5 ml or evaporate to just dryness and then take up in 0·5 ml distilled water.

**4.3.3** *Paper Chromatography*

**4.3.3.1** Take a Whatman No. 1 ( *see* **4.2.3** ) unwashed chromatographic paper and rule the starting line 2.5 cm from the bottom edge with a hard pencil. Spot two 1 $\mu$l spots with 1-$\mu$l pippette on the

paper 2·5 cm apart from each other, leaving atleast 2·5 cm margin, the first spot being of propionic acid standard solution and the second of unknown sample. Let the paper dry and clip it to a glass rod and suspend it in the chromatographic tank with 50 ml of mobile solvent in a trough. Do not saturate the tank with mobile solvent before inserting paper. Seal the glass cover with cellophane or other suitable tape and let it develop until solvent reaches 2·5 cm from top of paper. Remove the paper from the tank and let it air dry.

4.3.3.2 Spray chromogenic reagent on front side of the paper. Spraying should be uniform and rather heavy but not to the extent that chromogenic reagent runs or drips. Faint yellow spots indicate presence of propionic acid.

4.3.3.3 To intensify the acid spots, place paper in the atmosphere of ammonia fumes momentarily ( by placing 50 ml ammonium hydroxide in a 2-litre beaker and exposing to fumes by placing each end in beaker momentarily ), entire paper immediately turns green.

4.3.3.4 Remove paper from ammonia fumes, propionic acid gradually appears as red spots and presence of propionic acid in the sample may be determined by comparing its Rf value with that of standard propionic acid. Since colour of the acid is not stable, mark the spot with the pencil as soon as they are completely developed.

## 5. COLUMN CHROMATOGRAPHIC METHOD

### 5.1 Reagents

5.1.1 *Sodium Hydroxide* — 0·1 and 1N.

5.1.2 *Phosphotungstic Acid Solution* — 20 percent in distilled water.

5.1.3 *Crystalline Magnesium Sulphate* — $MgSO_4 .7H_2O$.

5.1.4 *Sulphuric Acid* — 1 N.

5.1.5 *Formic Acid* — 0·01 N.

5.1.6 *Alphamine Red R Indicator Solution* — 0·2 percent.

5.1.7 *Ammonium Hydroxide* — 1 N.

5.1.8 *Silicic Acid* — 100 mesh.

5.1.9 *Chloroform*

5.1.10 *Butyl Alcohol*

5.1.11 *Sulphuric Acid* — 50 percent

5.1.12 *Absolute Alcohol*

5.1.13 *Butanol in Chloroform* — 1 percent. Remove alcohol from chloroform by washing 3 times with water. Add 10 ml of n-butyl alcohol to 1 litre of washed chloroform in separating funnel, shake vigorously, add 25 ml of distilled water and shake again. Let it stand until the lower layer clears. Drain and discard the upper aqueous layer. Store it in contact with granular sodium sulphate.

5.1.14 *Cresol Red Indicator* — Dissolve 50 ml O - Cresol - sulphonphthalein 20 ml of absolute alcohol, add 1·3 ml of 0·1N sodium hydroxide and dilute to 50 ml with distilled water. Use 2 drops for each 25 ml of aqueous solution.

5.1.15 *Barium Hydroxide Standard Solution* — 0·01 N.

5.1.16 *Sodium Acetate — Sodium Chloride Solution* — Dissolve 12 g of sodium chloride and 25 g of sodium acetate in distilled water and dilute to 500 ml.

**5.2 Apparatus**

5.2.1 *Chromatographic Tube* — Approximately 15 × 250 mm.

5.2.2 *Rubber Bulb*

5.2.3 *Micro-funel* — 2-ml capacity.

5.2.4 *Steam Distillation Apparatus*

**5.3 Procedure**

5.3.1 *Sample Preparation* — Same as given in **4.3.1.**

5.3.2 *Distillation* — Weigh accurately 10 g of air dried sample and transfer it to a 150-ml distilling flask. Add 40 ml distilled water and 10 ml of 1 N sulphuric acid, mix thoroughly and add 10 ml of 20 percent phosphotungstic acid solution. Mix the contents well and add 40 g magnesium sulphate. Swirl the contents well and make the solution acidic to congo red paper with 50 percent sulphuric acid. Connect the condenser and steam generator and distill 200 ml in 35-40 minutes. Transfer the distillate to 400-600 ml beaker, add 10 ml 0·01 N formic acid, make alkaline to phenolphathalein with 1 N sodium hydroxide and evaporate to 5 ml. Transfer it into 225-ml glass stoppered test tube rinsing the beaker with three portions of distilled water. If insoluble matter adheres to the beaker, rinse with 1 N sulphuric acid. Make this solution alkaline to phenolphathalein and evaporate to just dryness by inserting the test tube in a steam bath.

5.3.3 *Chromatographic Separation*

5.3.3.1 *Preparation of partition column* — Take 5 g silicic acid in glazed porcelain evaporating dish and add 1 ml of alphamine Red R indicator solution and just enough 1 N ammonium hydroxide to give alkaline colour of the indicator ( 1 drop is enough ). Add maximum amount of distilled water that the silicic acid will hold without becoming sticky or agglomerating in the butyl alcohol-chloroform solution ( this amount shall be determined for each of silicic acid and usually varies from 50 to 75 percent of the weight of silicic acid ). Homogenize the mixture thoroughly in a pestle. Add 25 ml of 1 percent butyl alcohol in chloroform and mix to form a slurry that pours readily. Pour this slurry into a chromatographic tube containing a small cotton plug in the neck of the constricted end. To avoid air pockets, tilt the tube slightly while pouring. If air bubbles form while pouring, eliminate by stirring suspension in tube with a long glass rod. Clamp the tube vertically in ring stand. In the tube, insert a one-hole rubber stopper fitted with a glass tube bent to 90° angle and held in place by a Bunsen clamp against the pressure to be exerted. Connect the bent glass tube to a pressure source. Adjust the pressure to 5-10 psi ( 34·5 - 68·9 kPa ) so that excess solvent is forced through the column dropwise.

During removal of excess solvent, gel packs down. As the column packs down, particles of gel adhere to the wall of the tube, but eventually the gel leaves the wall of the tube relatively clean. This is the point of optimum density of the column, and the column is ready for use. Apply pressure until solvent reaches the surface of the column. If solvent passes below surface causing drying or 'cracking of column' or if air pockets are present extrude packing from the tube, reslurry with the solvent and repack the column.

5.3.3.2 *Preparation of standard propionic acid solution* — Prepare stock solution of propionic acid by diluting 5 ml of propionic acid to 250 ml with distilled water. Pipette 1 ml of stock solution into a 125-ml Erlenmeyer flask and titrate with 0·01 N sodium hydroxide, using cresol red as indicator, to pink colour which persists for 45 seconds.

$$\text{mg acid/ml standard solution} = \text{ml 0·01 N sodium hydroxide} \times \text{normality} \times F$$

where

$F = 7{\cdot}41$ for propionic acid.

5.3.3.3 *Preparation of known samples* — Pipette out 50 ml of standard solution into a 50-ml beaker and just neutralize with 0·01 N

sodium hydroxide solution, using phenolphthalein and add 10 drops in excess. Evaporate it to dryness on a steam bath.

**5.3.3.4** *Column separation* — To the dry residue add 2 ml of 10 percent butyl alcohol in chloroform solution and while stirring with glass rod add 50 percent sulphuric acid dropwise until the sodium salts are converted to free acids ( acid to congo red paper ) and add 1 g of anhydrous sodium sulphate. Place a 50-ml graduated cylinder under the column as receiver. Decant the supernatant onto column, pouring it slowly down the side of the tube without disturbing level surface of column. Apply pressure until solvent reaches surface of gel. Wash the beaker with 1 ml solvent and pour into column. Apply pressure until the solvent just disappears into sodium sulphate layer. Wash the beaker with another 1 ml of solvent, transfer to the column, wash the inside of the tube with 1 ml of solvent and apply pressure until the solvent just disappears into sodium sulphate layer. Fill the tube with the solvent and apply pressure. Once the band reaches the point 2-5 mm above the narrowest portion of constriction of tube, record the volume and remove the receiver.

Transfer the elute to a 125-ml Erlenmeyer flask, rinsing the cylinder with three 5-ml portions of distilled water. Add one drop of cresol red indicator and titrate with 0·1 N barium hydroxide solution. As its end point approaches, stopper flask and shake vigorously to completely extract acid from solvent phase. Correct titration for blank as follows. Collect 25 ml of butyl alcohol-chloroform mixture from column before the acid is transferred, add 15 ml boiled and cooled distilled water and titrate as above with 0·01 N barium hydroxide solution.

**5.4 Calculation** — Calculate the results for propionic acid as mg/100 g sample.

$$\text{Propionic acid, mg/100 g} = 7{\cdot}40 \times \text{ml } 0{\cdot}01 \text{ N barium hydroxide solution}$$

$$\text{Calcium propionate} = \text{propionic acid content} \times 1{\cdot}256$$

**5.5 Identification of Propionic Acid** — Acid separated in butyl alcohol-chloroform solution may be further identified by paper chromatography as described in **4.3.3.**

( *Continued from page* 2 )

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